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(54) Title: A NOVEL β 1- $>$ 6 N-ACETYLGLUCOSAMINYLTRANSFERASE, ITS ACCEPTOR MOLECULE, LEUKOSIALIN, AND A METHOD FOR CLONING PROTEINS HAVING ENZYMATIC ACTIVITY					
(57) Abstract					
The present invention provides a novel β 1- $>$ 6 N-acetylglucosaminyltransferase, which forms core 2 oligosaccharide structures in O-glycans, and a novel acceptor molecule, leukosialin, CD43, for core 2 β 1- $>$ 6 N-acetylglucosaminyltransferase activity. The amino acid sequences and nucleic acid sequences encoding these molecules, as well as active fragments thereof, also are disclosed. A method for isolating nucleic acid sequences encoding proteins having enzymatic activity is disclosed, using CHO cells that support replication of plasmid vectors having a polyoma virus origin of replication. A method to obtain a suitable cell line that expresses an acceptor molecule also is disclosed.					

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**A NOVEL β 1 \rightarrow 6 N-ACETYLGLUCOSAMINYLTRANSFERASE,
ITS ACCEPTOR MOLECULE, LEUKOSIALIN, AND
A METHOD FOR CLONING PROTEINS HAVING ENZYMATIC ACTIVITY**

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invention.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

10 This invention relates generally to the fields of
biochemistry and molecular biology and more specifically to
a novel human enzyme, UDP-GlcNAc:Gal β 1 \rightarrow 3GalNAc (GlcNAc to
GalNAc) β 1 \rightarrow 6 N-acetylglucosaminyltransferase (core 2 β 1 \rightarrow 6
N-acetylglucosaminyltransferase; C2GnT), and to a novel
15 acceptor molecule, leukosialin, CD43, for core 2 β 1 \rightarrow 6 N-
acetylglucosaminyltransferase action. The invention
additionally relates to DNA sequences encoding core 2 β 1 \rightarrow 6
N-acetylglucosaminyltransferase and leukosialin, to vectors
containing a C2GnT DNA sequence or a leukosialin DNA
20 sequence, to recombinant host cells transformed with such
vectors and to a method of transient expression cloning in
CHO cells for identifying and isolating DNA sequences
encoding specific proteins, using CHO cells expressing a
suitable acceptor molecule.

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BACKGROUND INFORMATION

Most O-glycosidic oligosaccharides in mammalian
glycoproteins are linked via N-acetylgalactosamine to the
hydroxyl groups of serine or threonine. These O-glycans
can be classified into 4 different groups depending on the
30 nature of the core portion of the oligosaccharides (see
Fig. 1). Although less well studied than N-glycans, O-
glycans likely have important biological functions.
Indeed, the presence of O-linked oligosaccharides with the

core 2 branch, Gal β 1 \rightarrow 3(GlcNAc β 1 \rightarrow 6)GalNAc, has been demonstrated in many biological processes.

Piller et al., J. Biol. Chem. 263:15146-15150 (1988) reported that human T-cell activation is associated with the conversion of core 1-based tetrasaccharides to core 2-based hexasaccharides on leukosialin, a major sialoglycoprotein present on human T lymphocytes (see also Fig. 1). A similar increase in hexasaccharides was observed in peripheral blood lymphocytes of patients suffering from T-cell leukemias (Saitoh et al., Blood 77:1491-1499 (1991)), myelogenous leukemias (Brockhausen et al., Cancer Res. 51:1257-1263 (1991)) and immunodeficiency due to AIDS and the Wiskott-Aldrich syndrome (Piller et al., J. Exp. Med. 173:1501-1510 (1991)). In these patients' lymphocytes, changes in the amount of hexasaccharides were caused by increased activity of either UDP-GlcNAc:Gal β 1 \rightarrow 3GalNAc (GlcNAc to GalNAc) 6- β -D-N-acetylglucosaminyltransferase (EC2.4.1.102) or core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase (Williams et al., J. Biol. Chem. 255:11253-11261 (1980)). Increased activity of core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase also was observed in metastatic murine tumor cell lines as compared to their parental, non-metastatic counterparts (Yousefi et al., J. Biol. Chem. 266:1772-1782 (1991)).

Increased complexity of the attached oligosaccharides increases the molecular weight of the glycoprotein. For example, leukosialin containing hexasaccharides has a molecular weight of ~135kDa, whereas leukosialin containing tetrasaccharides has a molecular weight of ~105kDa (Carlsson et al., J. Biol. Chem. 261:12779-12786 and 12787-12795 (1986)).

Fox et al., J. Immunol. 131:762-767 (1983) raised a monoclonal antibody, T305, against human T-lymphocytic leukemia cells. Sportsman et al., J. Immunol. 135:158-164

(1985) reported T305 binding was abolished by neuraminidase treatment, suggesting T305 binds to hexasaccharides. T305 specifically reacts with the high molecular weight form of leukosialin (Saitoh et al., supra, (1991)).

5 Previous studies indicated poly-*N*-acetyllactosamine repeats extend almost exclusively from the branch formed by the core 2 β 1 \rightarrow 6 *N*-acetylglucosaminyltransferase (Fukuda et al., J. Biol. Chem. 261:12796-12806 (1986)). Consistent with these
10 results, Yousefi et al., supra, (1991) demonstrated that the core 2 enzyme in metastatic tumor cells regulates the level of poly-*N*-acetyllactosamine synthesis in *O*-linked oligosaccharides.

Poly-*N*-acetyllactosamines are subject to a
15 variety of modifications, including the formation of the sialyl Le^x, NeuNAc2 \rightarrow 3Gal β 1 \rightarrow 4(Fuc α 1 \rightarrow 3)GlcNAc-, or the sialyl Le^a, NeuNAc2 \rightarrow 3Gal β 1 \rightarrow 3 (Fuc α 1 \rightarrow 4)GlcNAc-, determinants (Fukuda, Biochim. Biophys. Acta 780:119-150 (1985)). Such modifications are significant because these determinants,
20 which are present on neutrophils and monocytes, serve as ligands for E- and P-selectin present on endothelial cells and platelets, respectively (see, for example, Larsen et al., Cell 63:467-474 (1990)).

In addition, tumor cells often express a
25 significant amount of sialyl Le^x and/or sialyl Le^a on their cell surfaces. The interaction between E-selectin or P-selectin and these cell surface carbohydrates may play a role in tumor cell adhesion to endothelium during the metastatic process (Walz et al., supra, (1990)). Kojima et
30 al., Biochem. Biophys. Res. Commun. 182:1288-1295 (1992) reported that selectin-dependent tumor cell adhesion to endothelial cells was abolished by blocking *O*-glycan synthesis. Complex sulfated *O*-glycans also may serve as ligands for the lymphocyte homing receptor, L-selectin

(Imai et al., J. Cell Biol. 113:1213-1221 (1991)).

These reported observations establish core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase as a critical enzyme in O-glycan biosynthesis. The availability of core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase will allow the in vivo and in vitro production of specific glycoproteins having core 2 oligosaccharides and subsequent study of these variant O-glycans on cell-cell interactions. For example, core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase is a useful marker for transformed or cancerous cells. An understanding of the role of core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase in transformed and cancerous cells may elucidate a mechanism for the aberrant cell-cell interactions observed in these cells. In order to understand the control of expression of these oligosaccharides and their function, isolation of a cDNA clone for core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase is a prerequisite. However, the DNA sequence encoding core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase has not yet been reported.

Thus, a need exists for identifying the core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase and the DNA sequences encoding this enzyme. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention generally relates to a novel purified human $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase. A cDNA sequence encoding a 428 amino acid protein having $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase activity also is provided. The purified human $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase, or an active fragment thereof, catalyzes the formation of critical branches in O-glycans.

The invention further relates to a novel purified acceptor molecule, leukosialin, CD43, for core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase activity. The leukosialin cDNA encodes a novel variant leukosialin, which is created
5 by alternative splicing of the genomic leukosialin DNA sequence.

Isolated nucleic acids encoding either core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase or leukosialin are disclosed, as are vectors containing the nucleic acids and
10 recombinant host cells transformed with such vectors. The invention further provides methods of detecting such nucleic acids by contacting a sample with a nucleic acid probe having a nucleotide sequence capable of hybridizing with the isolated nucleic acids of the present invention.
15 The core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase and leukosialin amino acid and nucleic acid sequences disclosed herein can be purified from human cells or produced using well known methods of recombinant DNA technology.

The invention also discloses a method of
20 isolating nucleic acid sequences encoding proteins that have an enzymatic activity. Such a nucleic acid sequence is obtained by transfecting the nucleic acid, which is contained within a vector having a polyoma virus replication origin, into a Chinese hamster ovary (CHO) cell
25 line simultaneously expressing polyoma virus large T antigen and the acceptor molecule for the protein having an enzymatic activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the structures and biosynthesis
30 of O-glycans. Structures of O-glycan cores can be classified into 4 groups (core 1 to core 4), each of which is synthesized starting with GalNAc $\alpha 1 \rightarrow$ Ser/Thr. The core 1 structure is synthesized by the addition of a $\beta 1 \rightarrow 3$ Gal

residue to the GalNAc residue. The core 1 structure can be converted to core 2 by the addition of a $\beta 1 \rightarrow 6$ N-acetylglucosaminyl residue. This intermediate is usually converted to the hexasaccharide by sequential addition of 5 galactose and sialic acid residues (bottom right). The core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase and the linkage formed by the enzyme are indicated by a box. In certain cell types, the core 2 structure can be extended by the addition of N-acetyllactosamine ($\text{Gal} \beta 1 \rightarrow 4 \text{GlcNAc} \beta 1 \rightarrow 3$) repeats 10 to form poly-N-acetyllactosamine. In the absence of core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase, core 1 is converted to the monosialoform, then to the disialoform by sequential addition of $\alpha 2 \rightarrow 3$ - and $\alpha 2 \rightarrow 6$ -linked sialic acid residues (bottom left). Alternatively, core 3 can be synthesized by 15 the addition of a $\beta 1 \rightarrow 3$ N-acetylglucosaminyl residue to the GalNAc residue. Core 3 can be converted to core 4 by another $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase (top of figure).

Figure 2 depicts genomic DNA sequence (SEQ. ID. 20 NO. 1) and cDNA sequence (SEQ. ID. NO. 2) of leukosialin. The genomic sequence is numbered relative to the transcriptional start site. Exon 1 and exon 2 have been previously described. Exon 1' is newly identified here. In the isolated cDNA, exon 1' is immediately followed by 25 the exon 2 sequence. Deduced amino acids are presented under the coding sequence, which begins in exon 2 (SEQ. ID. NO. 3). A portion of the exon 2 sequence is shown.

Figure 3 establishes the ability of pGT/hCG to replicate in CHO cell lines expressing polyoma large T 30 antigen and leukosialin. In panel A, six clonal CHO cell lines were examined for replication of pcDNAI-based pGT/hCG (lanes 1-6). In panel B, replication of cell clone 5 (CHO-Py-leu), was further examined by treatment with increasing concentrations of DpnI and XhoI (lanes 2 and 3). Plasmid 35 DNA isolated from MOP-8 cells was used as a control (lane

1). Plasmid DNA was extracted using the Hirt procedure and samples were digested with XhoI and DpnI. In parallel, pGT/hCG plasmid purified from *E. coli* MC1061/P3 was digested with XhoI and DpnI (lane 7 in panel A and lane 4 in panel B) or XhoI alone (lane 8 in panel A and lane 5 in panel B). The arrow indicates the migration of plasmid DNA resistant to DpnI digestion. The arrowheads indicate plasmid DNA digested by DpnI.

Figure 4 shows the expression of T305 antigen expressed by pcDNAI-C2GnT. Subconfluent CHO-Py-leu cells were transfected with pcDNAI-C2GnT (panels A and B) or mock-transfected with pcDNAI (panels C and D). Sixty four hours after transfection, the cells were fixed, then incubated with mouse T305 monoclonal antibody followed by fluorescein isocyanate-conjugated sheep anti-mouse IgG (panels A, B and C). Two different areas are shown in panels A and B. Panel D shows a phase micrograph of the same field shown in panel C. Bar = 20 μ m.

Figure 5 depicts the cDNA sequence (SEQ. ID. NO. 4) and translated amino acid sequences (SEQ. ID. NO. 5) of core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase. The open reading frame and full-length nucleotide sequence of C2GnT are shown. The signal/membrane-anchoring domain is doubly underlined. The polyadenylation signal is boxed. Potential N-glycosylation sites are marked with asterisks. The sequences are numbered relative to the translation start site.

Figure 6 shows the expression of core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase mRNA in various cell types. Poly(A)⁺ RNA (11 μ g) from CHO-Py-leu cells (lane 1), HL-60 promyelocytes (lane 2), K562 erythrocytic cells (lane 3), and SP and L4 colonic carcinoma cells (lanes 4 and 5) was resolved by electrophoresis. RNA was transferred to a nylon membrane and hybridized with a radiolabeled fragment

of pPROTA-C2GnT. Migration of RNA size markers is indicated.

Figure 7 illustrates the construction of the vector encoding the protein A-C2GnT fusion protein. The 5 cDNA sequence corresponding to Pro³⁸ to His⁴²⁸ was fused in frame with the IgG binding domain of *S. aureus* protein A (bottom; SEQ. ID. NO. 6). The sequence includes the cleavable signal peptide, which allows secretion of the fused protein. The coding sequence is under control of the 10 SV40 promoter. The remainder of the vector sequence shown was derived from rabbit β -globin gene sequences, including an intervening sequence (IVS) and a polyadenylation signal (An).

DETAILED DESCRIPTION OF THE INVENTION

15 The present invention generally relates to a novel human core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase. The invention further relates to a novel method of transient expression cloning in CHO cells that was used to isolate the cDNA sequence encoding human core 2 β 1 \rightarrow 6 N- 20 acetylglucosaminyltransferase (C2GnT). The invention also relates to a novel human leukosialin, which is an acceptor molecule for core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase activity.

25 Cells generally contain extremely low amounts of glycosyltransferases. As a result, cDNA cloning based on screening using an antibody or a probe based on the glycosyltransferase amino acid sequence has met with limited success. However, isolation of cDNAs encoding various glycosyltransferases can be achieved by transient 30 expression of cDNA in recipient cells.

Successful application of the transient expression cloning method to isolate a cDNA sequence

encoding a glycosyltransferase requires an appropriate recipient cell line. Ideal recipient cells should not express the glycosyltransferase of interest. As a result, the recipient cells would normally lack the oligosaccharide structure formed by such a glycosyltransferase.

Expression of the cloned glycosyltransferase cDNA in the recipient cell line should result in formation of the specific oligosaccharide structure. The resultant oligosaccharide can be identified using a specific antibody or lectin that recognizes the structure. The recipient cell line also must support replication of an appropriate plasmid vector.

COS-1 cells initially appear to satisfy the requirements for using the transient expression method. COS-1 cells express SV40 large T antigen and support the replication of plasmid vectors harboring a SV40 replication origin (Gluzman et al., Cell 23:175-182 (1981)). Although COS-1 cells, themselves, express a variety of glycosyltransferases, COS-1 cells have been used to clone cDNA sequences encoding human blood group Lewis $\alpha 1 \rightarrow 3/4$ fucosyltransferase and murine $\alpha 1 \rightarrow 3$ galactosyltransferase (Kukowska-Latallo et al., Genes and Devel. 4:1288-1303 (1990); Larsen et al., Proc. Natl. Acad. Sci. USA 86:8227-8231 (1989)). Also, Goetz et al., Cell 63:175-182 (1990), utilized an antibody that inhibits E-selectin mediated adhesion to isolate a cDNA sequence encoding $\alpha 1 \rightarrow 3$ fucosyltransferase.

An attempt was made to use COS-1 cells to isolate cDNA clones encoding core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase. COS-1 cells were transfected using cDNA obtained from activated human T cells, which express the core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase. Transfected cells suspected of expressing core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase

in the transfected cells were identified by the presence of increased levels of the core 2 oligosaccharide structure formed by core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase activity. The presence of the core 2 structure was 5 identified using the monoclonal antibody, T305, which identifies a hexasaccharide on leukosialin. A clone expressing high levels of the T305 antigen was isolated and sequenced.

Surprisingly, transfection using COS-1 cells 10 resulted in the isolation of a cDNA clone encoding a novel variant of human leukosialin, which is the acceptor molecule for core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase activity. Examination of the cDNA sequence of the newly isolated leukosialin revealed the cDNA sequence was formed 15 as a result of alternative splicing of exons in the genomic leukosialin DNA sequence. Specifically, the newly isolated leukosialin is encoded by cDNA sequence containing a previously undescribed non-coding exon at the 5'-terminus (exon 1' in Figure 2; SEQ. ID. NO. 1 and SEQ. ID. NO. 2).

The unexpected result obtained using COS-1 cells 20 led to the development of a new transfection system to isolate a cDNA sequence encoding core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase. CHO cells, which do not normally express the T305 antigen, were transfected with 25 DNA sequences encoding human leukosialin and the polyoma virus large T antigen. A cell line, designated CHO-Py-leu, which expresses human leukosialin and polyoma virus large T antigen, was isolated.

CHO-Py-leu cells were used for transient 30 expression cloning of a cDNA sequence encoding core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase. CHO-Py-leu cells were transfected with cDNA obtained from human HL-60 promyelocytes. A plasmid, pcDNAI-C2Gnt, which directed expression of the T305 antigen, was isolated and the cDNA

insert was sequenced (see Figure 5; SEQ. ID. NO. 4). The 2105 base pair cDNA sequence encodes a putative 428 amino acid protein. The genomic DNA sequence encoding can be isolated using methods well known to those skilled in the art, such as nucleic acid hybridization using the core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase cDNA disclosed herein to screen, for example, a genomic library prepared from HL-60 promyelocytes.

An enzyme similar to the disclosed human core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase has been purified from bovine tracheal epithelium (Ropp et al., J. Biol. Chem. 266:23863-23871 (1991), which is incorporated herein by reference. The apparent molecular weight of the bovine enzyme is ~69kDa. In comparison, the predicted molecular weight of the polypeptide portion of core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase is ~50kDa. The deduced amino acid sequence of core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase reveals two to three potential N-glycosylation sites, suggesting N-glycosylation and O-glycosylation, or other post-translational modification, could account for the larger apparent size of the bovine enzyme.

Expression of the cloned C2GnT sequence, or a fragment thereof, directed formation of the specific O-glycan core 2 oligosaccharide structure. Although several cDNA sequences encoding glycosyltransferases have been isolated (Paulson and Colley, J. Biol. Chem. 264:17615-17618 (1989); Schachter, Curr. Opin. Struct. Biol. 1:755-765 (1991), which are incorporated herein by reference), C2GnT is the first reported cDNA sequence encoding an enzyme involved exclusively in O-glycan synthesis.

In O-glycans, $\beta 1 \rightarrow 6$ N-acetylglucosaminyl linkages may occur in both core 2, Gal $\beta 1 \rightarrow 3$ (GlcNAc $\beta 1 \rightarrow 6$)GalNAc, and core 4, GlcNAc $\beta 1 \rightarrow 3$ (GlcNAc $\beta 1 \rightarrow 6$)GalNAc, structures

(Brockhausen et al., Biochemistry 24:1866-1874 (1985), which is incorporated herein by reference. In addition, $\beta 1 \rightarrow 6$ N-acetylglucosaminyl linkages occur in the side chains of poly-N-acetyllactosamine, forming the I-structure 5 (Piller et al., J. Biol. Chem. 259:13385-13390 (1984), which is incorporated herein by reference), and in the side chain attached to α -mannose of the N-glycan core structure, forming a tetraantennary saccharide (Cummings et al., J. Biol. Chem. 257:13421-13427 (1982), which is incorporated 10 herein by reference). The enzymes responsible for these linkages all share the unique property that Mn^{2+} is not required for their activity.

Although it was originally suggested that these $\beta 1 \rightarrow 6$ N-acetylglucosaminyl linkages were formed by the same 15 enzyme (Piller at al., 1984), the present disclosure clearly demonstrates that the HL-60-derived core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase is specific for the formation only of O-glycan core 2. This result is consistent with a recent report demonstrating that myeloid cell lysates 20 contain the enzymatic activity associated with core 2, but not core 4, formation (Brockhausen et al., supra, (1991)).

Analysis of mRNA isolated from colonic cancer cells indicated core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase is expressed in these cells. Recent studies using affinity 25 absorption suggested at least two different $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferases were present in tracheal epithelium (Ropp et al., supra, (1991)). One of these transferases formed core 2, core 4, and I structures. Thus, at least one other $\beta 1 \rightarrow 6$ N- 30 acetylglucosaminyltransferase present in epithelial cells can form core 2, core 4 and I structures. Similarly, a $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase present in Novikoff hepatoma cells can form both core 2 and I structures (Koenderman et al., Eur. J. Biochem. 166:199-208 (1987), 35 which is incorporated herein by reference).

The acceptor molecule specificity of core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase is different from the specificity of the enzymes present in tracheal epithelium and Novikoff hepatoma cells. Thus, a family of $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferases can exist, the members of which differ in acceptor specificity but are capable of forming the same linkage. Members of this family are isolated from cells expressing $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase activity, using, for example, nucleic acid hybridization assays and studies of acceptor molecule specificity. Such a family was reported for the $\alpha 1 \rightarrow 3$ fucosyltransferases (Weston et al., J. Biol. Chem. 267:4152-4160 (1992), which is incorporated herein by reference).

The formation of the core 2 structure is critical to cell structure and function. For example, the core 2 structure is essential for elongation of poly-N-acetyllactosamine and for formation of sialyl Le^x or sialyl Le^y structures. Furthermore, the biosynthesis of cartilage keratan sulfate may be initiated by the core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase, since the keratan sulfate chain is extended from a branch present in core 2 structure in the same way as poly-N-acetyllactosamine (Dickenson et al., Biochem. J. 269:55-59 (1990), which is incorporated herein by reference). Keratan sulfate is absent in wild-type CHO cells, which do not express the core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase (Esko et al., J. Biol. Chem. 261:15725-15733 (1986), which is incorporated herein by reference). These structures are believed to be important for cellular recognition and matrix formation. The availability of the cDNA clone encoding the core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase will aid in understanding how the various carbohydrate structures are formed during differentiation and malignancy. Manipulation of the expression of the various carbohydrate structures by gene

transfer and gene inactivation methods will help elucidate the various functions of these structures.

The present invention is directed to a method for transient expression cloning in CHO cells of cDNA sequences encoding proteins having enzymatic activity. Isolation of human core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase is provided as an example of the disclosed method. However, the method can be used to obtain cDNA sequences encoding other proteins having enzymatic activity.

For example, lectins and antibodies reactive with other specific oligosaccharide structures are available and can be used to screen for glycosyltransferase activity. Also, CHO cell lines that have defects in glycosylation have been isolated. These cell lines can be used to study the activity of the corresponding glycosyltransferase (Stanley, Ann. Rev. Genet. 18:525-552 (1984), which is incorporated herein by reference). CHO cell lines also have been selected for various defects in cellular metabolism, loss of expression of cell surface molecules and resistance to cytotoxic drugs (see, for example, Malmström and Krieger, J. Biol. Chem. 266:24025-24030 (1991); Yayon et al., Cell 64:841-848 (1991), which are incorporated herein by reference). The approach disclosed herein should allow isolation of cDNA sequences encoding the proteins involved in these various cellular functions.

As used herein, the terms "purified" and "isolated" mean that the molecule or compound is substantially free of contaminants normally associated with a native or natural environment. For example, a purified protein can be obtained from a number of methods. The naturally-occurring protein can be purified by any means known in the art, including, for example, by affinity purification with antibodies having specific reactivity with the protein. In this regard, anti-core 2 β 1 \rightarrow 6 N-

acetylglucosaminyltransferase antibodies can be used to substantially purify naturally-occurring core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase from human HL-60 promyelocytes.

5 Alternatively, a purified protein of the present invention can be obtained by well known recombinant methods, utilizing the nucleic acids disclosed herein, as described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual 2d ed. (Cold Spring Harbor 10 Laboratory 1989), which is incorporated herein by reference, and by the methods described in the Examples below. Furthermore, purified proteins can be synthesized by methods well known in the art.

As used herein, the phrase "substantially the sequence" includes the described nucleotide or amino acid sequence and sequences having one or more additions, deletions or substitutions that do not substantially affect the ability of the sequence to encode a protein have a desired functional activity. In addition, the phrase encompasses any additional sequence that hybridizes to the disclosed sequence under stringent hybridization sequences. Methods of hybridization are well known to those skilled in the art. For example, sequence modifications that do not substantially alter such activity are intended. Thus, a 25 protein having substantially the amino acid sequence of Figure 5 (SEQ. ID. NO. 5) refers to core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase encoded by the cDNA described in Example IV, as well as proteins having amino acid sequences that are modified but, nevertheless, retain the 30 functions of core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase. One skilled in the art can readily determine such retention of function following the guidance set forth, for example, in Examples V and VI.

The present invention is further directed to active fragments of the human core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase protein. As used herein, an active fragment refers to portions of the protein that 5 substantially retain the glycosyltransferase activity of the intact core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase protein. One skilled in the art can readily identify active fragments of proteins such as core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase by comparing the activities 10 of a selected fragment with the intact protein following the guidance set forth in the Examples below.

As used herein, the term "glycosyltransferase activity" refers to the function of a glycosyltransferase to link sugar residues together through a glycosidic bond 15 to create critical branches in oligosaccharides. Glycosyltransferase activity results in the specific transfer of a monosaccharide to an appropriate acceptor molecule, such that the acceptor molecule contains oligosaccharides having critical branches. One skilled in 20 the art would understand the terms "enzymatic activity" and "catalytic activity" to generally refer to a function of certain proteins, such as the function of those proteins having glycosyltransferase activity.

As used herein, the term "acceptor molecule" 25 refers to a molecule that is acted upon by a protein having enzymatic activity. For example, an acceptor molecule, such as leukosialin, as identified by the amino acid sequence of Figure 2 (SEQ. ID. NO. 3), accepts the transfer of a monosaccharide due to glycosyltransferase activity. 30 An acceptor molecule, such as leukosialin, may already contain one or more sugar residues. The transfer of monosaccharides to an acceptor molecule, such as leukosialin, results in the formation of critical branches of oligosaccharides.

As used herein, the term "critical branches" refers to oligosaccharide structures formed by specific glycosyltransferase activity. Critical branches may be involved in various cellular functions, such as cell-cell recognition. The oligosaccharide structure of a critical branch can be determined using methods well known in the art, such as the method for determining the core 2 oligosaccharide structure, as described in Examples V and VI.

Relatedly, the invention also provides nucleic acids encoding the human core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase protein and leukosialin protein described above. The nucleic acids can be in the form of DNA, RNA or cDNA, such as the novel C2GnT cDNA of 2105 base pairs identified in Figure 5 (SEQ. ID. NO. 4) or the novel leukosialin cDNA identified in Figure 2 (SEQ. ID. NO. 2), for example. Such nucleic acids can also be chemically synthesized by methods known in the art, including, for example, the use of an automated nucleic acid synthesizer.

The nucleic acid can have substantially the nucleotide sequence of C2GnT, identified in Figure 5 (SEQ. ID. NO. 4), or leukosialin identified in Figure 2 (SEQ. ID. NO. 2). Portions of such nucleic acids that encode active fragments of the core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase protein or leukosialin protein of the present invention also are contemplated.

Nucleic acid probes capable of hybridizing to the nucleic acids of the present invention under reasonably stringent conditions can be prepared from the cloned sequences or by synthesizing oligonucleotides by methods known in the art. The probes can be labeled with markers according to methods known in the art and used to detect the nucleic acids of the present invention. Methods for

detecting such nucleic acids can be accomplished by contacting the probe with a sample containing or suspected of containing the nucleic acid under hybridizing conditions, and detecting the hybridization of the probe to 5 the nucleic acid.

The present invention is further directed to vectors containing the nucleic acids described above. The term "vector" includes vectors that are capable of expressing nucleic acid sequences operably linked to 10 regulatory sequences capable of effecting their expression. Numerous cloning vectors are known in the art. Thus, the selection of an appropriate cloning vector is a matter of choice. In general, useful vectors for recombinant DNA are often plasmids, which refer to circular double stranded DNA 15 loops such as pcDNAI or pcDSR α . As used herein, "plasmid" and "vector" may be used interchangeably as the plasmid is a common form of a vector. However, the invention is intended to include other forms of expression vectors that serve equivalent functions.

20 Suitable host cells containing the vectors of the present invention are also provided. Host cells can be transformed with a vector and used to express the desired recombinant or fusion protein. Methods of recombinant expression in a variety of host cells, such as mammalian, 25 yeast, insect or bacterial cells are widely known. For example, a nucleic acid encoding core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase or a nucleic acid encoding leukosialin can be transfected into cells using the calcium phosphate technique or other transfection methods, such as 30 those described in Sambrook et al., supra, (1989).

Alternatively, nucleic acids can be introduced into cells by infection with a retrovirus carrying the gene or genes of interest. For example, the gene can be cloned into a plasmid containing retroviral long terminal repeat

sequences, the C2Gnt DNA sequence or the leukosialin DNA sequence, and an antibiotic resistance gene for selection. The construct can then be transfected into a suitable cell line, such as PA12, which carries a packaging deficient provirus and expresses the necessary components for virus production, including synthesis of amphotrophic glycoproteins. The supernatant from these cells contain infectious virus, which can be used to infect the cells of interest.

10 Isolated recombinant polypeptides or proteins can be obtained by growing the described host cells under conditions that favor transcription and translation of the transfected nucleic acid. Recombinant proteins produced by the transfected host cells are isolated using methods set forth herein and by methods well known to those skilled in the art.

Also provided are antibodies having specific reactivity with the core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase protein or leukosialin protein of the present invention. Active fragments of antibodies, for example, Fab and Fab',₂ fragments, having specific reactivity with such proteins are intended to fall within the definition of an "antibody." Antibodies exhibiting a titer of at least about 1.5×10^5 , as determined by ELISA, are useful in the present invention.

The antibodies of the invention can be produced by any method known in the art. For example, polyclonal and monoclonal antibodies can be produced by methods described in Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor 1988), which is incorporated herein by reference. The proteins, particularly core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase or leukosialin of the present invention can be used as immunogens to generate such antibodies. Altered antibodies, such as chimeric,

humanized, CDR-grafted or bifunctional antibodies can also be produced by methods well known to those skilled in the art. Such antibodies can also be produced by hybridoma, chemical synthesis or recombinant methods described, for 5 example, in Sambrook et al., supra, (1989).

The antibodies can be used for determining the presence or purification of the core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase protein or the leukosialin protein of the present invention. With respect to the 10 detecting of such proteins, the antibodies can be used for in vitro or in vivo methods well known to those skilled in the art.

Finally, kits useful for carrying out the methods of the invention are also provided. The kits can contain 15 a core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase protein, antibody or nucleic acid of the present invention and an ancillary reagent. Alternatively, the kit can contain a leukosialin protein, antibody or nucleic acid of the present invention and an ancillary reagent. An ancillary 20 reagent may include diagnostic agents, signal detection systems, buffers, stabilizers, pharmaceutically acceptable carriers or other reagents and materials conventionally included in such kits.

A cDNA sequence encoding core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase was isolated and core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase activity was determined. This is the first report of transient expression cloning using CHO cells expressing polyoma large T antigen. The 25 following examples are intended to illustrate but not limit 30 the present invention.

EXAMPLE IEXPRESSION CLONING IN COS-1 CELLS OF THE cDNA FOR THE PROTEIN CARRYING THE HEXASACCHARIDES

COS-1 cells were transfected with a cDNA library,
5 pcDSR α -2F1, constructed from poly(A)⁺ RNA of activated T lymphocytes, which express the core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase (Yokota et al., Proc. Natl. Acad. Sci. USA 83:5894-5898 (1986); Piller et al., supra, (1988), which are incorporated herein by reference). COS-1
10 cells support replication of the pcDSR α constructs, which contain the SV40 replication origin. Transfected cells were selected by panning using monoclonal antibody T305, which recognizes sialylated branched hexasaccharides (Piller et al., supra, (1991); Saitoh et al., supra,
15 (1991)). Methods referred to in this example are described in greater detail in the examples that follow.

Following several rounds of transfection, one plasmid, pcDSR α -leu, directing high expression of the T305 antigen was identified. The cloned cDNA insert was
20 isolated and sequenced, then compared with other reported sequences. The newly isolated cDNA sequence was nearly identical to the sequence reported for leukosialin, except the 5'-flanking sequences were different (Pallant et al., Proc. Natl. Acad. Sci. USA 86:1328-1332 (1989), which is
25 incorporated herein by reference).

Comparison of the cloned cDNA sequence with the genomic leukosialin DNA sequence revealed the start site of the cDNA sequence is located 259 bp upstream of the transcription start site of the previously reported
30 sequence (Figure 2; compare Exon 1' and Exon 1) (Shelley et al., Biochem. J. 270:569-576 (1990); Kudo and Fukuda, J. Biol. Chem. 266:8483-8489 (1991), which are incorporated herein by reference). A consensus splice site was

identified at the exon-intron junction of the newly identified 122 bp exon 1' in pcDSR α -leu (Breathnach and Chambon, Ann. Rev. Biochem. 50:349-383 (1981), which is incorporated herein by reference). This splice site is
5 followed by the exon 2 sequence.

These results indicate the T305 antibody preferentially binds to branched hexasaccharides attached to leukosialin. Indeed, a small amount of the hexasaccharides (approximately 8% of the total) was
10 detected in O-glycans isolated from control COS-1 cells. T305 binding is similar to anti-M and anti-N antibodies, which recognize both the glycan and polypeptide portions of erythrocyte glycoprotein, glycophorin (Sadler et al., J. Biol. Chem. 254: 2112-2119 (1979), which is incorporated
15 herein by reference). These observations are consistent with reports that only leukosialin strongly reacted with T305 in Western blots of leukocyte cell extracts, even though leukocytes also express other glycoproteins, such as CD45, that must also contain the same hexasaccharides
20 (Piller et al., supra, (1991); Saitoh et al., supra, (1991)).

EXAMPLE II

ESTABLISHMENT OF CHO CELL LINES THAT STABLY EXPRESS POLYOMA VIRUS LARGE T ANTIGEN AND LEUKOSIALIN

25 T305 preferentially binds to branched hexasaccharides attached to leukosialin. Such hexasaccharides are not present on the erythropoietin glycoprotein produced in CHO cells, although the glycoprotein does contain the precursor tetrasaccharide
30 (Sasaki et al., J. Biol. Chem. 262:12059-12076 (1987), which is incorporated herein by reference). T305 antigen also is not detectable in CHO cells transiently transfected with pcDSR α -leu. In order to screen for the presence of a

cDNA clone expressing core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase activity, a CHO cell line expressing both leukosialin and polyoma large T antigen was established (see, for example, Heffernan and Dennis Nucl. Acids Res. 19:85-92 (1991), which is incorporated herein by reference).

Vectors: A plasmid vector, pPSVE1-PyE, which contains the polyoma virus early genes under the control of the SV40 early promoter, was constructed using a modification of the method of Muller et al., Mol. Cell. Biol. 4:2406-2412 (1984), which is incorporated herein by reference. Plasmid pPSVE1 was prepared using pPSG4 (American Type Culture Collection 37337) and SV40 viral DNA (Bethesda Research laboratories) essentially as described by Featherstone et al., Nucl. Acids Res. 12:7235-7249 (1984), which is incorporated herein by reference. Following EcoRI and HincII digestion of plasmid pPyLT-1 (American Type Culture Collection 41043), a DNA sequence containing the carboxy terminal coding region of polyoma virus large T antigen was isolated. The HincII site was converted to an EcoRI site by blunt-end ligation of phosphorylated EcoRI linkers (Stratagene). Plasmid pPSVE1-PyE was generated by inserting the carboxy-terminal coding sequence for large T antigen into the unique EcoRI site of plasmid pPSVE1.

Plasmid pZIPNEO-leu was constructed by introducing the EcoRI fragment of PEER-3 cDNA, which contains the complete coding sequence for human leukosialin, into the unique EcoRI site of plasmid pZIPNEO (Cepko et al., Cell 37:1053-1063 (1984), which is incorporated herein by reference). Plasmid structures were confirmed by restriction mapping and by sequencing the construction sites. pZIPNEO was kindly provided by Dr. Channing Der.

Transfection: CHODG44 cells were grown in 100 mm tissue

culture plates. When the cells were 20% confluent, they were co-transfected with a 1:4 molar ratio of pZIPNEO-leu and pPSVEL-PyE using the calcium phosphate technique (Graham and van der Eb, Virology 52:456-467 (1973), which 5 is incorporated herein by reference). Transfected cells were isolated and maintained in medium containing 400 µg/ml G-418 (active drug).

Leukosialin expression: The total pool of G418-resistant transfectants was enriched for human leukosialin 10 expressing cells by a one-step panning procedure using anti-leukosialin antibodies and goat anti-rabbit IgG coated panning dishes (Sigma) (Carlsson and Fukuda J. Biol. Chem. 261:12779-12786 (1986), which is incorporated herein by reference). Clonal cell lines were obtained by limiting 15 dilution. Six clonal cell lines expressing human leukosialin on the cell surface were identified by indirect immunofluorescence and isolated for further studies (Williams and Fukuda J. Cell Biol. 111:955-966 (1990), which is incorporated herein by reference).

20 Polyoma virus-mediated replication: The ability of the six clonal cell lines to support polyoma virus large T antigen-mediated replication of plasmids was assessed by determining the methylation status of transfected plasmids containing a polyoma virus origin of replication (Muller et al., supra, 1984; Heffernan and Dennis, supra, 1991). Plasmid pGT/hCG contains a fused β 1 \rightarrow 4 galactosyltransferase and human chorionic gonadotropin α -chain DNA sequence inserted in plasmid pcDNAI, which contains a polyoma virus 25 replication origin (Aoki et al., Proc. Natl. Acad. Sci., USA 89, 4319-4323 (1992), which is incorporated herein by reference).

Plasmid pGT/hCG was isolated from methylase-positive *E. coli* strain MC1061/P3 (Invitrogen), which methylates the adenine residues in the DpnI recognition

site, "GATC". The methylated DpnI recognition site is susceptible to cleavage by DpnI. In contrast, the DpnI recognition site of plasmids replicated in mammalian cells is not methylated and, therefore, is resistant to DpnI 5 digestion.

Methylated plasmid pGT/hCG was transfected by lipofection into each of the six selected clonal cell lines expressing leukosialin. After 64 hr, low molecular weight plasmid DNA was isolated from the cells using the method of 10 Hirt, J. Mol. Biol. 26:365-369 (1967), which is incorporated herein by reference. Isolated plasmid DNA was digested with XhoI and DpnI (Stratagene), subjected to electrophoresis in a 1% agarose gel, and transferred to nylon membranes (Micron Separations Inc., MA).

15 A 0.4 kb SmaI fragment of the $\beta 1 \rightarrow 4$ galactosyltransferase DNA sequence of pGT/hCG was radiolabeled with [32 P]dCTP using the random primer method (Feinberg and Vogelstein, Anal. Biochem. 132:6-13 (1983), which is incorporated herein by reference). Hybridization 20 was performed using methods well-known to those skilled in the art (see, for example, Sambrook et al., supra, (1989)). Following hybridization, the membranes were washed several times, including a final high stringency wash in 0.1 x SSPE, 0.1% SDS for 1 hr at 65°C, then exposed to Kodak X-AR 25 film at -70°C.

Four of the six clones tested supported replication of the pcDNAI-based plasmid, pGT/hCG (Fig. 3.A., lanes 1, 3, 4 and 5). MOP-8 cells, a 3T3 cell line transformed by polyoma virus early genes (Muller et al., 30 supra, (1984)), expresses endogenous core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase activity and was used as a control for the replication assay (Fig. 3.B., lane 1). One clonal cell line that supported pGT/hCG replication, CHO-Py-leu (Fig. 3.A., lane 5; Fig. 3.B., lanes 2 and 3) and

expressed a significant amount of leukosialin, was selected for further studies. pGT/hCG was kindly provided by Dr. Michiko Fukuda.

EXAMPLE III

5 ISOLATION OF A cDNA SEQUENCE DIRECTING EXPRESSION OF THE
HEXASACCHARIDE ON LEUKOSIALIN

Poly(A)⁺ RNA was isolated from HL-60 promyelocytes, which contain a significant amount of the core 2 β 1 \rightarrow 6N-acetylglucosaminyltransferase (Saitoh et al., 10 supra, (1991)). A cDNA expression library, pcDNAI-HL-60, was prepared (Invitrogen) and the library was screened for clones directing the expression of the T305 antigen.

Plasmid DNA from the pcDNAI-HL-60 cDNA library was transfected into CHO-Py-leu cells using a modification 15 of the lipofection procedure, described below (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987), which is incorporated herein by reference). CHO-Py-leu cells were grown in 100 mm tissue culture plates. When the cells were 20% confluent, they were washed twice with Opti-MEM I 20 (GIBCO). Fifty μ g of lipofectin reagent (Bethesda Research Laboratories) and 20 μ g of purified plasmid DNA were each diluted to 1.5 ml with Opti-MEM I, then mixed and added to the cells. After incubation for 6 hr at 37°C, the medium was removed, 10 ml of complete medium was added and 25 incubation was continued for 16 hr at 37°C. The medium was then replaced with 10 ml of fresh medium.

Following a 64 hr period to allow transient expression of the transfected plasmids, the cells were detached in PBS/5mM EDTA, pH7.4, for 30 min at 37°C, 30 pooled, centrifuged and resuspended in cold PBS/10mM

EDTA/5% fetal calf serum, pH7.4, containing a 1:200 dilution of ascites fluid containing T305 monoclonal antibody. The cells were incubated on ice for 1 hr, then washed in the same buffer and panned on dishes coated with 5 goat anti-mouse IgG (Sigma) (Wysocki and Sato Proc. Natl. Acad. Sci. USA 75:2844-2848 (1978); Seed & Aruffo Proc. Natl. Acad. Sci. USA 84:3365-3369 (1987), which are incorporated herein by reference). T305 monoclonal antibody was kindly provided by Dr. R.I. Fox, Scripps 10 Research Foundation, La Jolla, CA.

Plasmid DNA was recovered from adherent cells by the method of Hirt, supra, (1967), treated with DpnI to eliminate plasmids that had not replicated in transfected cells, and transformed into *E. coli* strain MC1061/P3. 15 Plasmid DNA was then recovered and subjected to a second round of screening. *E. coli* transformants containing plasmids recovered from this second enrichment were plated to yield 8 pools of approximately 500 colonies each. Replica plates were prepared using methods well-known to 20 those skilled in the art (see, for example, Sambrook et al., supra, (1989)).

The pooled plasmid DNA was prepared from replica plates and transfected into CHO-Py-leu cells. The transfectants were screened by panning. One plasmid pool 25 was selected and subjected to three subsequent rounds of selection. One plasmid, pcDNAI-C2GnT, which directed the expression of the T305 antigen, was isolated. CHO-Py-leu cells transfected with pcDNAI-C2GnT express the antigen recognized by T305, whereas CHO-Py-leu cells transfected 30 with pcDNAI are negative for T305 antigen (Fig. 4). These results show pcDNAI-C2GnT directs the expression of a new determinant on leukosialin that is recognized by T305 monoclonal antibody. This determinant is the branched hexa saccharide sequence, 35 NeuNAc α 2 \rightarrow 3Gal β 1 \rightarrow 3(NeuNAc α 2 \rightarrow 3Gal β 1 \rightarrow 4 GlcNAc β 1 \rightarrow 6)GalNAc.

EXAMPLE IVCHARACTERIZATION OF C2GnT

DNA sequence: The cDNA insert in plasmid pcDNAI-C2GnT was sequenced by the dideoxy chain termination method using 5 Sequenase version 2 reagents (United States Biochemicals) (Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467 (1977), which is incorporated herein by reference). Both strands were sequenced using 17-mer synthetic oligonucleotides, which were synthesized as the sequence of 10 the cDNA insert became known.

Plasmid pcDNAI-C2GnT contains a 2105 base pair insert (Fig. 5). The cDNA sequence ends 1878 bp downstream of the putative translation start site. A polyadenylation signal is present at nucleotides 1694-1699. The 15 significance of the large number of nucleotides between the polyadenylation signal and the beginning of the polyadenyl chain is not clear. However, this sequence is A/T rich.

Deduced amino acid sequence: The cDNA insert in plasmid pcDNAI-C2GnT encodes a single open reading frame in 20 the sense orientation with respect to the pcDNAI promoter (Fig. 5). The open reading frame encodes a putative 428 amino acid protein having a molecular mass of 49,790 daltons.

Hydropathy analysis indicates the predicted 25 protein is a type II transmembrane molecule, as are all previously reported mammalian glycosyltransferases (Schachter, supra, (1991)). In this topology, a nine amino acid cytoplasmic NH₂-terminal segment is followed by a 23 amino acid transmembrane domain flanked by basic amino acid 30 residues. The large COOH-terminus consists of the stem and catalytic domains and presumably faces the lumen of the Golgi complex.

The putative protein contains three potential N-glycosylation sites (Fig. 5, asterisks). However, one of these sites contains a proline residue adjacent to asparagine and is not likely utilized in vivo.

5 No matches were obtained when the C2GnT cDNA sequence and deduced amino acid sequence were compared with sequences listed in the PC/Gene 6.6 data bank. In particular, no homology was revealed between the deduced amino acid sequence of C2GnT and other
10 glycosyltransferases, including N-acetylglucosaminyltransferase I (Sarkar et al., Proc. Natl. Acad. Sci. USA 88:234-238 (1991), which is incorporated herein by reference).

mRNA expression: Poly(A)⁺ RNA was prepared using a kit
15 (Stratagene) and resolved by electrophoresis on a 1.2% agarose/2.2 M formaldehyde gel, and transferred to nylon membranes (Micro Separations Inc., MA) using methods well-known to those skilled in the art (see, for example, Sambrook et al., supra, (1989)). Membranes were probed
20 using the EcoRI insert of pPROTA-C2GnT (see below) radiolabeled with [³²P]dCTP by the random priming method (Feinberg and Vogelstein, supra, (1983)). Hybridization was performed in buffers containing 50% formamide for 24 hr at 42°C (Sambrook et al., supra, (1989)). Following
25 hybridization, filters were washed several times in 1xSSPE/0.1% SDS at room temperature and once in 0.1xSSPE/0.1% SDS at 42°C, then exposed to Kodak X-AR film at -70°C.

Fig. 6 compares the level of core 2 $\beta 1 \rightarrow 6$ N-
30 acetylglucosaminyltransferase mRNA isolated from HL-60 promyelocytes, K562 erythroleukemia cells, and poorly metastatic SP and highly metastatic L4 colonic carcinoma cells. The major RNA species migrates at a size essentially identical to the ~2.1 kb C2GnT cDNA sequence.

The same result is observed for HL-60 cells and the two colonic cell lines, which apparently synthesize the hexasaccharides. In addition, two transcripts of -3.3 kb and 5.4 kb in size were detected in these cell lines. The 5 two larger transcripts may result from differential usage of polyadenylation signals.

No hybridization occurred with poly(A)⁺ RNA isolated from K562 cells, which lack the hexasaccharide, but synthesize the tetrasaccharide (Carlsson et al., supra, 10 (1986)), which is incorporated herein by reference. Similarly, no hybridization was observed for poly(A)⁺ RNA isolated from CHO-Py-leu cells (Fig. 6, lane 1).

EXAMPLE V

15 EXPRESSION OF ENZYMATICALLY ACTIVE β 1 \rightarrow 6 N-
ACETYLGLUCOSAMINYLTRANSFERASE

In order to confirm that C2GnT cDNA encodes for core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase, enzymatic activity was examined in CHO-Py-leu cells transfected with pcDNAI or pcDNAI-C2GnT. Following a 64 hr period to allow 20 transient expression, cell lysates were prepared and core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase activity was measured.

N-acetylglucosaminyltransferase assays were performed essentially as described by Saitoh et al., supra, 25 (1991), Yousefi et al., supra, (1991), and Lee et al., J. Biol. Chem. 265:20476-20487 (1990), which is incorporated herein by reference. Each reaction contained 50 mM MES, pH7.0, 0.5 μ Ci of UDP-[³H]GlcNAc in 1 mM UDP-GlcNAc, 0.1 M GlcNAc, 10 mM Na₂EDTA, 1mM of acceptor and 25 μ l of either 30 cell lysate, cell supernatant or IgG-Sepharose matrix in a total reaction volume of 50 μ l.

Reactions were incubated for 1 hr at 37°C, then processed by C18 Sep-Pak chromatography (Waters) (Palcic et al., J. Biol. Chem. 265:6759-6769 (1990), which is incorporated herein by reference). Core 2 and core 4 β 1 \rightarrow 6 5 N -acetylglucosaminyltransferase were assayed using the acceptors p-nitrophenyl Gal β 1 \rightarrow 3GalNAc and p-nitrophenyl GlcNAc β 1 \rightarrow 3GalNAc, respectively (Toronto Research Chemicals).

10 UDP-GlcNAc: α -Man β 1 \rightarrow 6 N -acetylglucosaminyltransferase(V) was assayed using the acceptor GlcNAc β 1 \rightarrow 2Man α 1 \rightarrow 6Glc- β -O-(CH₂)₈CH₃. The blood group I enzyme, UDP-GlcNAc:GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4GlcNAc (GlcNAc to Gal) β 1 \rightarrow 6 N -acetylglucosaminyltransferase, was assayed using GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 6Man α 1 \rightarrow 6Man β 1 \rightarrow O-(CH₂)₈COOCH₃, 15 or Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow O-(CH₂)₈CH₃, as acceptors (Gu et al., J. Biol. Chem. 267:2994-2999 (1992), which is incorporated herein by reference). Synthetic acceptors were kindly provided by Dr. Olé Hindsgaul, University of Alberta, Canada.

20 Results of these assays are shown in Table I. Assuming transfection efficiency of the cells is approximately 20-30%, the level of enzymatic activity directed by cells transfected with pcDNAI-C2GnT is roughly equivalent to the level observed in HL-60 cells.

TABLE I

Core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase activity in CHO-Py-leu cell extracts transfected with pcDNAI or pcDNAI-C2GnT.

5

	Vector	Core 2 β 1 \rightarrow 6 GlcNAc transferase activity (pmol/mg of protein/hr)
	pcDNAI	n.d.
10	pcDNAI-C2GnT	764

CHO-Py-leu cells were transfected with pcDNAI or pcDNAI-C2GnT, as described in the specification. Endogenous activity was measured in the absence of acceptor and subtracted from values determined in the presence of added acceptor. Gal β 1 \rightarrow 3GalNAc α -p-nitrophenyl was used as an acceptor. n.d. = not detectable. For comparison, the core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase activity measured in HL-60 cells under identical conditions was 3228 pmol/mg of protein per hr.

In order to unequivocally establish that C2GnT cDNA sequence encodes core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase, plasmid, pPROTA-C2GnT was constructed containing the DNA sequence encoding the putative catalytic domain of core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase fused in frame with the signal peptide and IgG binding domain of *S. aureus* protein A (Fig. 7). The putative catalytic domain is contained in a 1330 bp fragment of the C2GnT cDNA that encodes amino acid residues 38 to 428. Plasmid pPROTA was kindly provided by Dr. John B. Lowe.

The polymerase chain reaction (PCR) was used to insert EcoRI recognition sites on either side of the 1330 bp sequence in pcDNAI-C2GnT DNA. PCR was performed using the synthetic oligonucleotide primers 5'-TTTGAATTCCCTGAATTGTAAGTGTCAGACAC-3' (SEQ. ID. NO. 6) and 5'-TTTGAATTCGCAGAAACCATGCAGCTCTGA-3' (SEQ. ID. NO. 7)

(EcoRI recognition sites underlined). The EcoRI sites allowed direct, in-frame insertion of the fragment into the unique EcoRI site of plasmid pPROTA (Sanchez-Lopez et al., *J. Biol. Chem.* 263:11892-11899 (1988), which is 5 incorporated herein by reference).

The nucleotide sequence of the insert as well as the proper orientation were confirmed by DNA sequencing using the primers described above for cDNA sequencing. Plasmid pPROTA-C2GnT allows secretion of the fusion protein 10 from transfected cells and binding of the secreted fusion protein by insolubilized immunoglobulins.

Either pPROTA or pPROTA-C2GnT was transfected into COS-1 cells. Following a 64 hr period to allow transient expression, cell supernatants were collected 15 (Kukowska-Latallo et al., *supra*, (1990)). Cell supernatants were cleared by centrifugation, adjusted to 0.05% Tween 20 and either assayed directly for core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase activity or used in IgG-Sepharose (Pharmacia) binding studies. For the latter 20 assay, supernatants (10 ml) were incubated batchwise with approximately 300 μ l of IgG-Sepharose for 4 hr at 4°C. The matrices were then extensively washed and used directly for glycosyltransferase assays.

No core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase 25 activity was detected in the medium of COS-1 cells transfected with the control plasmid, pPROTA. Similarly, no enzymatic activity was associated with IgG-Sepharose beads. In contrast, a significant level of core 2 β 1 \rightarrow 6 N-acetylglucosaminyltransferase activity was detected in the 30 medium of COS-1 cells transfected with pPROTA-C2GnT. The activity also associated with the IgG-Sepharose beads (Table II). No activity was detected in the supernatant following incubation of the supernatant with IgG-Sepharose.

TABLE II

Determination of Enzymatic Activities Directed by pPROTA-C2GnT.

	Acceptors and linkages formed	Radioactivity (cpm) with (+) and without (-) acceptor
		- +
5		
10	GlcNAc β 1 6 Gal β 1 \rightarrow 3GalNAc (core 2-GnT)	109 1048
15	GlcNAc β 1 6 GlcNAc β 1 \rightarrow 3GalNAc (core 4-GnT)	111 113
20	GlcNAc β 1 6 GlcNAc β 1 \rightarrow 2Man (GnTV)	118 115
25	GlcNAc β 1 6 GlcNAc β 1 \rightarrow 3Gal (I-GnT)	111 113
30	GlcNAc β 1 6 Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3Gal (I-GnT)	99 96

COS-1 cells were transfected with pPROTA-C2GnT and the conditioned media were incubated with IgG-Sepharose. The proteins bound to the IgG-Sepharose were assayed for β 1 \rightarrow 6 N-acetylglucosaminyltransferase activity by using appropriate acceptors. The linkages formed are indicated by italics. Similar results were obtained in three independent experiments.

EXAMPLE VIDETERMINATION OF C2GnT SPECIFICITY

Four types of $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase linkages have been reported, 5 including core 2 and core 4 in O-glycans, I-antigen and a branch attached to mannose that forms tetraantennary N-glycans (see Table II). In order to determine whether these different structures are also synthesized by the cloned C2GnT cDNA sequence, enzymatic activity was 10 determined using five different acceptors.

As shown in Table II, the fusion protein was only active with the acceptor for core 2 formation. The same was true when the formation of $\beta 1 \rightarrow 6$ N-acetylglucosaminyl linkage to internal galactose residues was examined (Table 15 II, see structure at bottom). This result precludes the likelihood that the enzyme encoded by the C2GnT cDNA sequence may add N-acetylglucosamine to a non-reducing terminal galactose. The HL-60 core 2 $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase is exclusively responsible 20 for the formation of the GlcNAc $\beta 1 \rightarrow 6$ branch on Gal $\beta 1 \rightarrow 3$ GalNAc.

Although the invention has been described with reference to the disclosed embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, 25 the invention is limited only by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: LA JOLLA CANCER RESEARCH FOUNDATION

(ii) TITLE OF INVENTION: A NOVEL BETA1-6
N-ACETYLGLUCOSAMINYLTRANSFERASE, ITS ACCEPTOR MOLECULE,
LEUKOSIALIN AND A METHOD FOR CLONING PROTEINS HAVING
ENZYMATIC ACTIVITY

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CAMPBELL AND FLORES
(B) STREET: 4370 LA JOLLA VILLAGE DRIVE, SUITE 700
(C) CITY: SAN DIEGO
(D) STATE: CALIFORNIA
(E) COUNTRY: USA
(F) ZIP: 92122

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE: 30 September 1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: KONSKI, ANTOINETTE F.
(B) REGISTRATION NUMBER: 34,202
(C) REFERENCE/DOCKET NUMBER: FP-LJ 9756

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 619-535-9001
(B) TELEFAX: 619-535-8949

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 900 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 841..900

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 91..192
(D) OTHER INFORMATION: /note= "EXON 1' IS LOCATED IN BOTH
GENOMIC AND cDNA. IN THE cDNA EXON 1' IS
IMMEDIATELY FOLLOWED BY EXON 2."

(ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 359..428
 (D) OTHER INFORMATION: /note= "EXON 1 IS LOCATED IN GENOMIC DNA"

(ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 193..806
 (D) OTHER INFORMATION: /note= "THIS SEGMENT OF NUCLEIC ACID CONSTITUTES INTRON SEQUENCE OF THE cDNA"

(ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 807..900
 (D) OTHER INFORMATION: /note= "EXON 2 IS LOCATED IN BOTH GENOMIC AND cDNA. IN THE cDNA EXON 2 IMMEDIATELY FOLLOWS EXON 1".

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTGGGGACCA CAAATGCAAA GGAAACCACC CTCCCCTCCC ACCTCCTCCT CTGCACCCCTT	60
GAGTTCTCAG GCTCACATTC CCACCACCCA CCTCTGAGCC CAGCCCTCCC TAGCATCACC	120
ACTTCCATCC CATTCCCTCAG CCAAGAGCCA GGAATCCTGA TTCCAGATCC CACGCTTCCC	180
TGCCTCCCTC AGGTGAGCCC CAGACCCCCA GGCACCCCGC TGGCCCTGA AGGAGCAGGT	240
GATGGTGCTG TCTTCGCCCA GCAGCTGTGG GAGCAGGCCG GTGGGGCAGG ATGGAGGGGT	300
GGGTGGGGTG GGTGGAGCCA GGGCCCAC TT CCTTCCCT TGGGGCCCTG TCCTTCCCAG	360
TCTTGGCCCA GCCTCGGGAG GTGGTGGAGT GACCTGGCCC CAGTGCTGCG TCCTTATCAG	420
CCGAGCCGGT AAGAGGGTGA GACTTGGTGG GGTAGGGCC TCAGTGGGCC TGGGAATGTG	480
CCTGTGGCTT GAAAAGACTC TGACAGGTTA TGATGGGAAG AGATTGGGAG CCATTGGGCT	540
GCACAGGGTC AGGGAAAGGCC AGGAGGGCT GGTCACTGCT GGAATCTAAG CTGCTGAGGC	600
TGGAGGGAGC CTCAGGATGG GGCTGATGGG GGAGCTGCCA GCATCTGTT CTCTGTCATT	660
TCTGATAACA GTAAAAGCCA GCATGGAAAA AACCGTTAAA CCGCAGGTTG GGCCTGGCCG	720
TTGGCAGGGA ACTGGGCAGA GGGGAGGCC GGCCAGGTCC TCCGGCAACT CCCGCGTGT	780
CTGCTTCTCC GGCTGCCAC CTGCAGGTCC CAGCTCTTGC TCCTGCCGT TTGCCTGGAA	840
ATG GCC ACG CTT CTC CTT CTC CTT GGG GTG CTG GTG GTA AGC CCA GAC	888
Met Ala Thr Leu Leu Leu Leu Gly Val Val Val Ser Pro Asp	
1 5 10 15	
GCT CTG GGG AGC	900
Ala Leu Gly Ser	
20	

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ala	Thr	Leu	Leu	Leu	Leu	Gly	Val	Leu	Val	Val	Ser	Pro	Asp
1														
									10					15
Ala	Leu	Gly	Ser											
			20											

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2105 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 220..1504

(ix) FEATURE:

- (A) NAME/KEY: polyA_signal
- (B) LOCATION: 1913..1918

(ix) FEATURE:

- (A) NAME/KEY: misc_signal
- (B) LOCATION: 248..314
- (D) OTHER INFORMATION: /standard_name= "SIGNAL/MEMBRANE-ANCHORING DOMAIN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTGAAGTGCT	CAGAATGGGG	CAGGATGTCA	CCTGGAATCA	GCACTAAGTG	ATTCAGACTT	60
TCCTTACTTT	TAAATGTGCT	GCTCTTCATT	TCAAGATGCC	GTTGCAGCTC	TGATAATGC	120
AAACTGACAA	CCTTCAAGGC	CACGACGGAG	GGAAAATCAT	TGGTGCTTGG	AGCATAGAAG	180
ACTGCCCTTC	ACAAAGGAAA	TCCCTGATTA	TTGTTTGAA	ATG CTG AGG ACG TTG		234
				Met Leu Arg Thr Leu		
				1	5	
CTG CGA AGG AGA CTT TTT TCT TAT CCC ACC AAA TAC TAC TTT ATG GTT						282
Leu Arg Arg Arg Leu Phe Ser Tyr Pro Thr Lys Tyr Tyr Phe Met Val	10	15	20			
CTT GTT TTA TCC CTA ATC ACC TTC TCC GTT TTA AGG ATT CAT CAA AAG						330
Leu Val Leu Ser Leu Ile Thr Phe Ser Val Leu Arg Ile His Gln Lys	25	30	35			
CCT GAA TTT GTA AGT GTC AGA CAC TTG GAG CTT GCT GGG GAG AAT CCT						378
Pro Glu Phe Val Ser Val Arg His Leu Glu Leu Ala Gly Glu Asn Pro	40	45	50			

AGT AGT GAT ATT AAT TGC ACC AAA GTT TTA CAG GGT GAT GTA AAT GAA Ser Ser Asp Ile Asn Cys Thr Lys Val Leu Gln Gly Asp Val Asn Glu 55 60 65	426
ATC CAA AAG GTA AAG CTT GAG ATC CTA ACA GTG AAA TTT AAA AAG CGC Ile Gln Lys Val Lys Leu Glu Ile Leu Thr Val Lys Phe Lys Lys Arg 70 75 80 85	474
CCT CGG TGG ACA CCT GAC GAC TAT ATA AAC ATG ACC AGT GAC TGT TCT Pro Arg Trp Thr Pro Asp Asp Tyr Ile Asn Met Thr Ser Asp Cys Ser 90 95 100	522
TCT TTC ATC AAG AGA CGC AAA TAT ATT GTA GAA CCC CTT AGT AAA GAA Ser Phe Ile Lys Arg Arg Lys Tyr Ile Val Glu Pro Leu Ser Lys Glu 105 110 115	570
GAG GCG GAG TTT CCA ATA GCA TAT TCT ATA GTG GTT CAT CAC AAG ATT Glu Ala Glu Phe Pro Ile Ala Tyr Ser Ile Val Val His His Lys Ile 120 125 130	618
GAA ATG CTT GAC AGG CTG CTG AGG GCC ATC TAT ATG CCT CAG AAT TTC Glu Met Leu Asp Arg Leu Leu Arg Ala Ile Tyr Met Pro Gln Asn Phe 135 140 145	666
TAT TGC GTT CAT GTG GAC ACA AAA TCC GAG GAT TCC TAT TTA GCT GCA Tyr Cys Val His Val Asp Thr Lys Ser Glu Asp Ser Tyr Leu Ala Ala 150 155 160 165	714
GTG ATG GCC ATC GCT TCC TGT TTT AGT AAT GTC TTT GTG GCC AGC CGA Val Met Gly Ile Ala Ser Cys Phe Ser Asn Val Phe Val Ala Ser Arg 170 175 180	762
TTG GAG AGT GTG GTT TAT GCA TCG TGG AGC CGG GTT CAG GCT GAC CTC Leu Glu Ser Val Val Tyr Ala Ser Trp Ser Arg Val Gln Ala Asp Leu 185 190 195	810
AAC TGC ATG AAG GAT CTC TAT GCA ATG AGT GCA AAC TGG AAG TAC TTG Asn Cys Met Lys Asp Leu Tyr Ala Met Ser Ala Asn Trp Lys Tyr Leu 200 205 210	858
ATA AAT CTT TGT GGT ATG GAT TTT CCC ATT AAA ACC AAC CTA GAA ATT Ile Asn Leu Cys Gly Met Asp Phe Pro Ile Lys Thr Asn Leu Glu Ile 215 220 225	906
GTC AGG AAG CTC AAG TTG TTA ATG GGA GAA AAC AAC CTG GAA ACG GAG Val Arg Lys Leu Lys Leu Leu Met Gly Glu Asn Asn Leu Glu Thr Glu 230 235 240 245	954
AGG ATG CCA TCC CAT AAA GAA GAA AGG TGG AAG AAG CGG TAT GAG GTC Arg Met Pro Ser His Lys Glu Glu Arg Trp Lys Lys Arg Tyr Glu Val 250 255 260	1002
GTT AAT GGA AAG CTG ACA AAC ACA GGG ACT GTC AAA ATG CTT CCT CCA Val Asn Gly Lys Leu Thr Asn Thr Gly Thr Val Lys Met Leu Pro Pro 265 270 275	1050
CTC GAA ACA CCT CTC TTT TCT GGC AGT GCC TAC TTC GTG GTC AGT AGG Leu Glu Thr Pro Leu Phe Ser Gly Ser Ala Tyr Phe Val Val Ser Arg 280 285 290	1098
GAG TAT GTG GGG TAT GTA CTA CAG AAT GAA AAA ATC CAA AAG TTG ATG Glu Tyr Val Gly Tyr Val Leu Gln Asn Glu Lys Ile Gln Lys Leu Met 295 300 305	1146
GAG TGG GCA CAA GAC ACA TAC AGC CCT GAT GAG TAT CTC TGG GCC ACC Glu Trp Ala Gln Asp Thr Tyr Ser Pro Asp Glu Tyr Leu Trp Ala Thr 310 315 320 325	1194

40

ATC CAA AGG ATT CCT GAA GTC CCG GGC TCA CTC CCT GCC AGC CAT AAG Ile Gln Arg Ile Pro Glu Val Pro Gly Ser Leu Pro Ala Ser His Lys 330 335 340	1242
TAT GAT CTA TCT GAC ATG CAA GCA GTT GCC AGG TTT GTC AAG TGG CAG Tyr Asp Leu Ser Asp Met Gln Ala Val Ala Arg Phe Val Lys Trp Gln 345 350 355	1290
TAC TTT GAG GGT GAT GTT TCC AAG GGT GCT CCC TAC CCG CCC TGC GAT Tyr Phe Glu Gly Asp Val Ser Lys Gly Ala Pro Tyr Pro Pro Cys Asp 360 365 370	1338
GGA GTC CAT GTG CGC TCA GTG TGC ATT TTC GGA GCT GGT GAC TTG AAC Gly Val His Val Arg Ser Val Cys Ile Phe Gly Ala Gly Asp Leu Asn 375 380 385	1386
TGG ATG CTG CGC AAA CAC CAC TTG TTT GCC AAT AAG TTT GAC GTG GAT Trp Met Leu Arg Lys His His Leu Phe Ala Asn Lys Phe Asp Val Asp 390 395 400 405	1434
GTT GAC CTC TTT GCC ATC CAG TGT TTG GAT GAG CAT TTG AGA CAC AAA Val Asp Leu Phe Ala Ile Gln Cys Leu Asp Glu His Leu Arg His Lys 410 415 420	1482
GCT TTG GAG ACA TTA AAA CAC T GACCATTACG GGCAATTTA TGAACAAGAA Ala Leu Glu Thr Leu Lys His 425	1534
GAAGGATACA CAAAACGTAC CTTATCTGTT TCCCCCTTCCT TGTCAGCGTC GGGAAAGATGG	1594
TATGAAGTCC TCTTGCGGC AGGGACTCTA GTAGATCTTC TTGTCAGAGA AGCTGCATGG	1654
TTTCTGCAGA GCACAGTTAG CTAGAAAGGT GATAGCATTA AATGTTCATC TAGAGTTAAT	1714
AGTGGGAGGA GTAAAGGTAG CCTTGAGGCC AGAGCAGGTA GCAAGGCATT GTGGAAAGAG	1774
GGGACCAGGG TGGCTGGGGAGA AGAGGCCGAT GCATAAAAGTC AGCCTGTTCC AAGTGCTCAG	1834
GGACTTAGCA AAATGAGAAG ATGTGACCTG TGCCAAAATCT ATTTTGAGAA TTTTAAATGT	1894
GACCATTTCCTGTTGATGAA TAAACTTACA GCAACAAATA ATCAAAGATA CAATTAATCT	1954
GATATTATAT TTGTTGAAAT AGAAATTGAA TTGTACTATA AATGATTTT GTAAAATAATT	2014
TATATTCTGC TCTAATACTG TACTGTGTAG TGTGTCTCCG TATGTCATCT CAGGGAGCTT	2074
AAAATGGGCT TGATTTAACAA TTGAAAAAAA A	2105

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 428 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Leu Arg Thr Leu Leu Arg Arg Leu Phe Ser Tyr Pro Thr Lys	
1 5 10 15	

Tyr Tyr Phe Met Val Leu Val Leu Ser Leu Ile Thr Phe Ser Val Leu	
20 25 30	

41

Arg Ile His Gln Lys Pro Glu Phe Val Ser Val Arg His Leu Glu Leu
 35 40 45

Ala Gly Glu Asn Pro Ser Ser Asp Ile Asn Cys Thr Lys Val Leu Gln
 50 55 60

Gly Asp Val Asn Glu Ile Gln Lys Val Lys Leu Glu Ile Leu Thr Val
 65 70 75 80

Lys Phe Lys Lys Arg Pro Arg Trp Thr Pro Asp Asp Tyr Ile Asn Met
 85 90 95

Thr Ser Asp Cys Ser Ser Phe Ile Lys Arg Arg Lys Tyr Ile Val Glu
 100 . 105 110

Pro Leu Ser Lys Glu Glu Ala Glu Phe Pro Ile Ala Tyr Ser Ile Val
 115 120 125

Val His His Lys Ile Glu Met Leu Asp Arg Leu Leu Arg Ala Ile Tyr
 130 135 140

Met Pro Gln Asn Phe Tyr Cys Val His Val Asp Thr Lys Ser Glu Asp
 145 150 155 160

Ser Tyr Leu Ala Ala Val Met Gly Ile Ala Ser Cys Phe Ser Asn Val
 165 170 175

Phe Val Ala Ser Arg Leu Glu Ser Val Val Tyr Ala Ser Trp Ser Arg
 180 185 190

Val Gln Ala Asp Leu Asn Cys Met Lys Asp Leu Tyr Ala Met Ser Ala
 195 200 205

Asn Trp Lys Tyr Leu Ile Asn Leu Cys Gly Met Asp Phe Pro Ile Lys
 210 215 220

Thr Asn Leu Glu Ile Val Arg Lys Leu Lys Leu Leu Met Gly Glu Asn
 225 230 235 240

Asn Leu Glu Thr Glu Arg Met Pro Ser His Lys Glu Glu Arg Trp Lys
 245 250 255

Lys Arg Tyr Glu Val Val Asn Gly Lys Leu Thr Asn Thr Gly Thr Val
 260 265 270

Lys Met Leu Pro Pro Leu Glu Thr Pro Leu Phe Ser Gly Ser Ala Tyr
 275 280 285

Phe Val Val Ser Arg Glu Tyr Val Gly Tyr Val Leu Gln Asn Glu Lys
 290 295 300

Ile Gln Lys Leu Met Glu Trp Ala Gln Asp Thr Tyr Ser Pro Asp Glu
 305 310 315 320

Tyr Leu Trp Ala Thr Ile Gln Arg Ile Pro Glu Val Pro Gly Ser Leu
 325 330 335

Pro Ala Ser His Lys Tyr Asp Leu Ser Asp Met Gln Ala Val Ala Arg
 340 345 350

Phe Val Lys Trp Gln Tyr Phe Glu Gly Asp Val Ser Lys Gly Ala Pro
 355 360 365

Tyr Pro Pro Cys Asp Gly Val His Val Arg Ser Val Cys Ile Phe Gly
 370 375 380

42

Ala Gly Asp Leu Asn Trp Met Leu Arg Lys His His Leu Phe Ala Asx
 385 390 395 400
 Lys Phe Asp Val Asp Val Asp Leu Phe Ala Ile Gln Cys Leu Asp Glx
 405 410 415
 His Leu Arg His Lys Ala Leu Glu Thr Leu Lys His
 420 425

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTTGAATTCC CCTGAATTTG TAAGTGTAG ACAC

34

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTTGAATTCG CAGAAACCAT GCAGCTTC TGA

33

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..15
- (D) OTHER INFORMATION: /note= "PROTEIN A - C2GNT FUSION PROTEIN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGG AAT TCC CCT GAA
 Gly Asn Ser Pro Glu
 1 5

15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Asn Ser Pro Glu
1 5

CLAIMS

We claim:

1. A purified human protein or an active fragment thereof having $\beta 1 \rightarrow 6$ N-5 acetylglucosaminyltransferase activity.
2. The purified protein of claim 1, wherein said activity is that of UDP-GlcNAc:Gal $\beta 1 \rightarrow 3$ GalNAc (GlcNAc to GalNAc) $\beta 1 \rightarrow 6$ N-acetylglucosaminyltransferase.
3. The purified protein of claim 2, wherein 10 said protein has a relative molecular weight of about 50 kD.
4. An isolated nucleic acid encoding the human protein or active fragment thereof of claim 1.
5. A vector containing the nucleic acid of 15 claim 4.
6. The vector of claim 5, wherein said vector is a plasmid.
7. The vector of claim 5, wherein said vector is pcDNAI-C2GnT.
- 20 8. A host cell containing the vector of claim 5.
9. A purified human protein or a fragment thereof that is an acceptor molecule, said acceptor molecule being acted upon by the protein of claim 2 having activity which exclusively forms core 2 oligosaccharide 5 structures in O-glycans.

10. The acceptor molecule of claim 9, wherein said acceptor molecule is leukosialin, CD43.

11. An isolated nucleic acid encoding the acceptor molecule of claim 9.

12. A vector containing the nucleic acid of claim 11.

13. The vector of claim 12, wherein said vector is a plasmid.

14. The vector of claim 12, wherein said vector is pcDSR α -leu.

15. A host cell containing the vector of claim 12.

16. A method of obtaining from a cell line, which does not normally contain a protein having catalytic activity or an acceptor molecule for said protein, a nucleic acid encoding said protein having catalytic activity comprising:

a. transfected said cell line with a DNA sequence encoding the acceptor molecule, wherein the acceptor molecule is stably expressed in the cell line;

10 b. transfected said cell line with a cDNA library containing said nucleic acid in a vector, wherein proteins encoded by the transfected cDNA are transiently expressed;

c. screening the transfected cells for expression of said protein having catalytic activity; and

15 d. isolating the nucleic acid encoding the protein having catalytic activity.

17. The vector of claim 16, wherein said vector replicates in the transfected cell line.

18. The vector in claim 17, wherein said vector is a plasmid.

19. The vector of claim 16, wherein said vector contains a viral replication origin.

20. The vector of claim 19, wherein said replication origin is the polyoma virus replication origin.

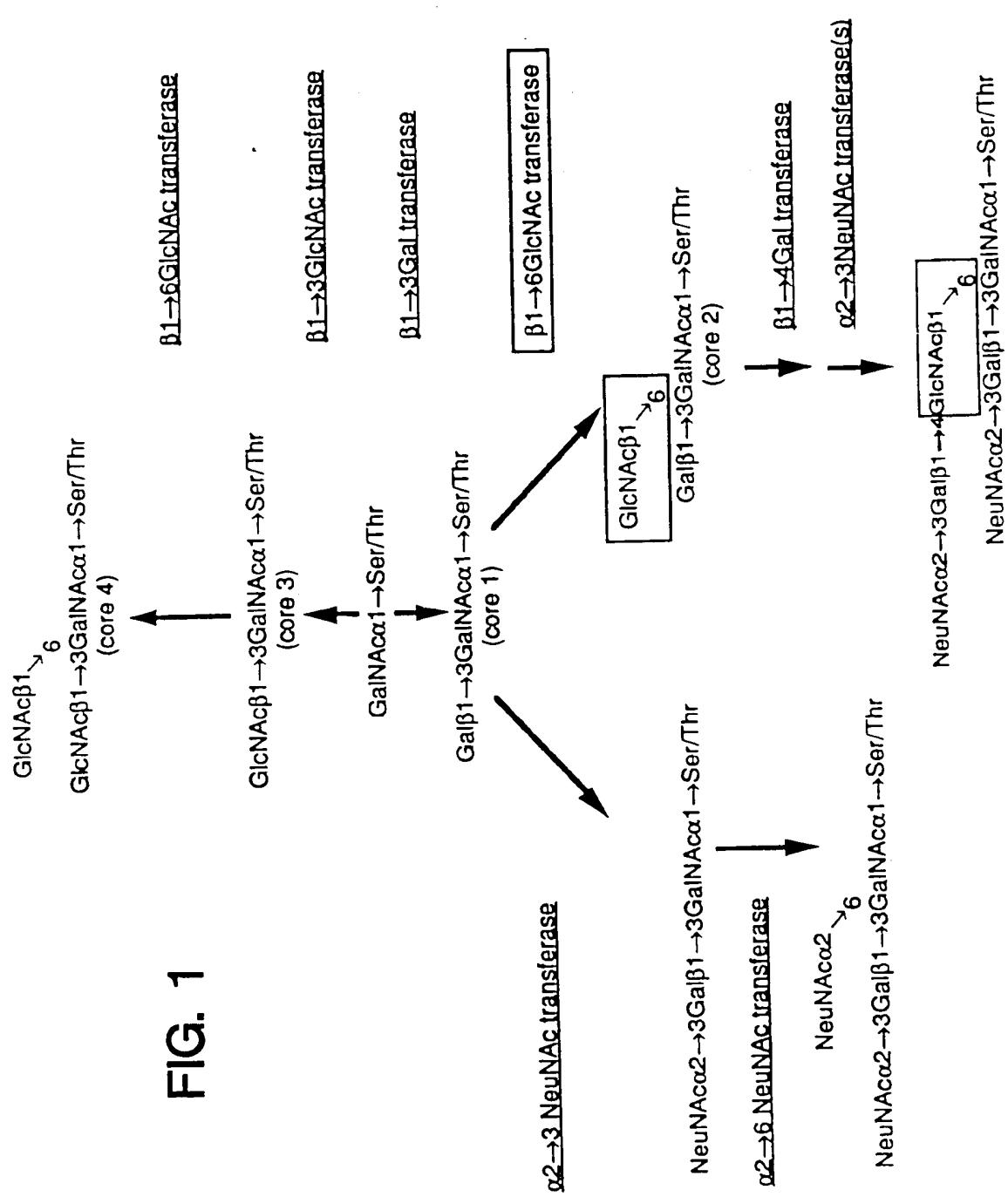
21. The cell line of claim 16, wherein said cell line supports replication of a vector.

22. The cell line of claim 16, wherein said cell line expresses polyoma virus large T antigen.

23. The cell line of claim 16, wherein said cell line is the Chinese hamster ovary cell line.

24. The cell line of claim 23, wherein said cell line is CHO-Py-leu.

25. A method of isolating a polypeptide having catalytic activity that forms core 2 oligosaccharide structures in O-glycans, said method comprising growing the host cell of claim 8 under conditions which favor expression of a nucleic acid encoding said polypeptide, and isolating said polypeptide so produced.
5



Exon1' <u>TTGGGACCAATGCAAAGGAAACCACCCCTCCCACCTCCCACCCACCTCTGAGCCAGCCCTCCCTAGCATCACC</u> <u>GAGTTCTCAGGCTCACATTCCCACCAACCCACTCTGAGCCAGCCCTCCCTAGCATCACC</u> <u>ACTTCATCCATTCTCAGCCAAGAGCCAGGAATCCTGATTCAAGATCCCACGCCCTCCC</u> <u>TGCCTCCCTCAAGTGAACCCCCAGAACCCCAGGGCACCCCGCTGCCCTGAAGGAGCAGGT</u> <u>GATGGTGCTGTCTGCCAGCAGCTGTGGAGCAGGCCACTCTTCCCTGGGCCCTGGGGAGGT</u> <u>GGGTGGGGTGGTGGAGCCAGGGCCACTCTTCCCTGGGCCCTGGGGAGGT</u> <u>ICTTGGCCCAGGCCCTGGGAGGTGGAGTGTGACCTGGGCCCTAGTGGCCTGGGAATGTG</u> <u>CCGAGCCGGTAAGAGGGTGAGACTCTGGGGTAGGGCTCAGTGGCCTGGGAATGTG</u> <u>CCTGGCTTGAAAAGACTCTGACAGGTTATGATGGGAAGAGATTGGAGCCATTGGCT</u> <u>GCACAGGGTCAGGGAAAGGCCAGGAGGGGCTGGTCACTGCTGGAATCTAAAGCTGCTGAGGC</u> <u>TCTGATAAACAGTAAAGCCAGCATGGAAAACCCGTTAACCCGAGGTTGGCCTGGCCG</u> <u>TTGGCAAGGGAAAGTGGCAGAGGGGGCCAGGTGGCTGGGAGCTGGGAGCTGGCTGGTGT</u> <u>CTGCTCTCCGGCTGCCACCTGCAGCTCCAGCTTCCCTGGGGTGGCTGGCTGGAA</u> <u>ATGGCCACGGCTTCTCCCTGGGGTGGCTGGCTGGCTGGCTGGGAGC</u> Exon2 <u>M A T L R L G V L V S P D A L G S (20)</u>	-299 -239 -179 -119 -59 2 62 122 182 242 302 362 422 482 542
---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

FIG. 2

1 2 3 4 5 6 7 8

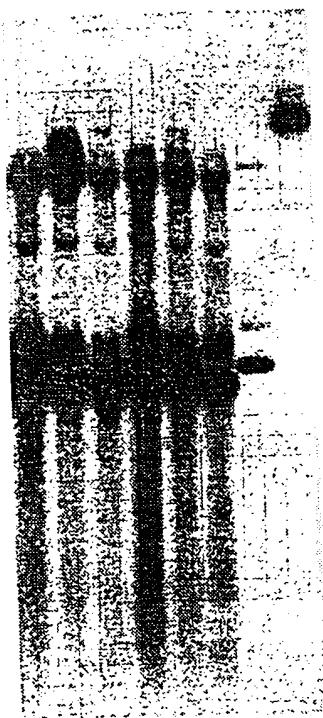


FIG. 3A

1 2 3 4 5

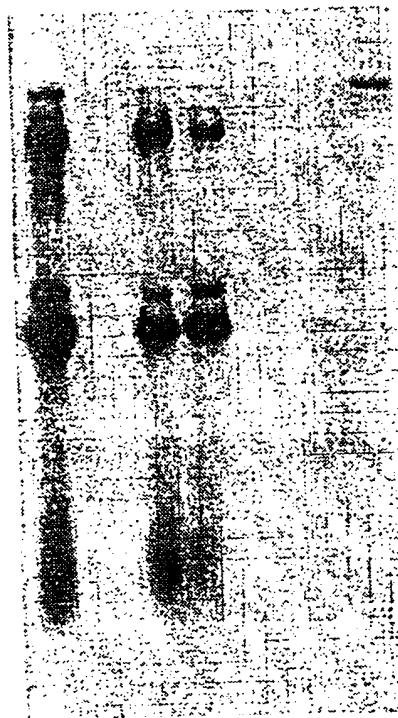


FIG. 3B



FIG. 4B

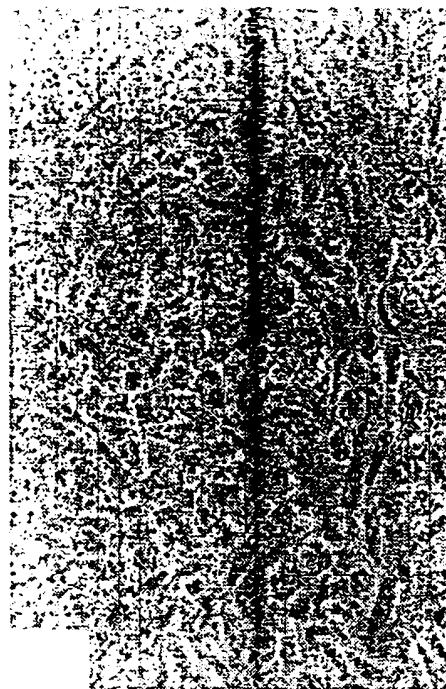


FIG. 4D



FIG. 4A



FIG. 4C

-219 GTGAAAGTGCCTGATAATGCAAACCTGACAACCTTCAGGGCAAGGATGTACCCGGAAATCAGGACTAAGTGATTGACTTCCCTTACTTTAAATGTCGCTCTCATTTCAAGATGCG
 -121
 CGTTGCAGCTCTGATAATGCAAACCTGACAACCTTCAGGGCAAGGATGTACCCGGAAATCAGGACTAAGTGATTGACTTCCCTTACTTTAAATGTCGCTCTCATTTCAAGATGCG
 M L R T L L R R F S Y P T K Y F M V L V L S L I T F S V L R 1 H Q K P E F 40
 ATGCTGAGGACGTTGCTGGAAGGAGACTTTTCTTATCCCACCAAATACTACTTTAAGGTCTGTTTATCCCTTAICACCTCTCCGTTTAAGGATTCAAAAGCCTGAATT
 120
 V S V R H L E N P S D I * C T K V L Q G D V N E I Q K V K L E I L T V 80
 GIAAGTGTAGACACTGGAGCTTGTGGAGAATCCTAGTAGTGTATAATTGGACCCAAGGTTTACAGGGTGTGTTAAAGGTTAAAGGAAATCCAAAAGGTTAAAGGAAAT
 240
 V S V R H L E N P S D I * C T K V L Q G D V N E I Q K V K L E I L T V 80
 AAATTTAAAGGCCCTGGGACACCTGACGACTATAAACATGACCAAGGACTGTTCTCATCAAGAGACGGAAATATAATTGGTAGAAACCCTTATAGTAAAGGAAGGGGAG
 K F K R P R W T P D Y I N M T S D C S F I K R R K Y I V E P L S K E E A E 120
 TTCTCAAATGGCATATTCTATAGTGGTCATCACAGATTGAAATGCTTGAGGGCTGCTGAGGGCTCAGAATTCTATGGCTCAGAATTCTATGGCTCAGAATT
 480
 F P I A Y S I V V H H K I E M L D R L R A I Y M P Q N F Y C V H V D T K S E D 160
 TCCTATTAGCTGGAGTGGCATGGCATGGCTTCTGTTTAGTAATGTCITTGCCAGCCGATTGGAGAGTGTGGTTATGCATGTGGACACAAAATCCGAGGAT
 600
 S Y L A A V M G I A S C F S N V F V A S R L E S V V A S W S R V Q A D L N C M 200
 AGGATCTCTATGCAAATGAGTGGAAACTGGTACTTGTATAAAACCAACTGAAATTGGATTTCCCAITAAAGCTCAAGTTGTTAAATGGGAGAAAC
 720
 K D L Y A M S A N W K Y L I N L C G M D F P I K T N L E I V R K L K L M G E N 240

SUBSTITUTE SHEET

6/8

AACCTGGAAACGGAGGATGCCATCCCCATAAGAAGAAAGGTGGAAAGAAGCTGACAAAATGGAAAGCTGACAAACAGGGACTGICAAATGCTCCACTCGAAACA
 N L E T E R M P S H K E E R W K R Y E V N G K L T N T G T V K M L P P L E T 840
 280
 CCTCTCTTCTGGCAGTCCTACTCTGTGTCAGTAGGGAGATGTGGGATGATGTTACTACAGAATGAAAATCCAAGTGTGATGGAGTGGCACACACATACGCCCTGATGAG
 P L F S G S A Y F V V S R E Y V L Q N E K I Q K L M E W A Q D T Y S P D E 960
 320
 TATCTCTGGCCACCATCCAAAGGATTCCCTGAAGTCCCAGGGCTCACTGCCAGCCATAAGIATGATCTATCTGACATIGCAAGCAGTTGCCAGGTGTCAGTACTTTGAG
 Y L W A T I Q R I P E V P G S L P A S H K Y D L S D M Q A V A R F V K W Q Y F E 1080
 360
 GGTGATGTTCCAAAGGGTGCCTCCTACCCGCCCTGCAGTGGAGTCCATGTCGGCCTCAGTGCGCAGTGGCAACTGGATIGCTGCCAAACACCACCTGTTGCCAAT
 G D V S K G A P Y P C D G V H V R S V C I F G A G D L N W M L R K H L F A N 1200
 400
 AAGTTTGACGTGGATGTTGACCTCTTGCATCCAGTGGATGAGCATAAAAGCATTGGAGACATTAACACTGACCAATTACGGCAATTATGAACAAAGAAGAAGG
 K F D V D L F A I Q C L D E H L R H K A L E T L K H end 1320
 428
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 GCAGAGCACAGTTAGCTAGAAAGGTGATAAGCATTAAATGTTCATCTAGAGTTAAATAGTGGAGGAGTAAGGTAGGCCAGGGTAGCAAGGCTTGGAAAGGGGAC 1440
 1560
 CAGGGTGGCTGGGAAAGAGGCCATGCTCAAGTCAGCTGTCAGGFACTTAGCAAAATGAGAAGATGIGACCTGIGCCAAACTAATTIGAGAATTITAAATGTGACCA
 1680
 TTTTCTGGTATGATAAAACTTACAGCAACAAATAATCAAATAATCAAATAATCTGATATTATTTGTTGAAATAGAAATTGTACTATAAAATGATTTTGTAATTTATAT
 1800
 TCTGCTTAATACTGTACTGTGTAGTGTCTCGTATGTCAGGGAGCTCTCAAGGGACTGTCAGCTCGTATGTCAGGGAGCTAAATGGGCTGATTAAACATTGAAAMAAA 1886

SUBSTITUTE SHEET

FIG. 5B

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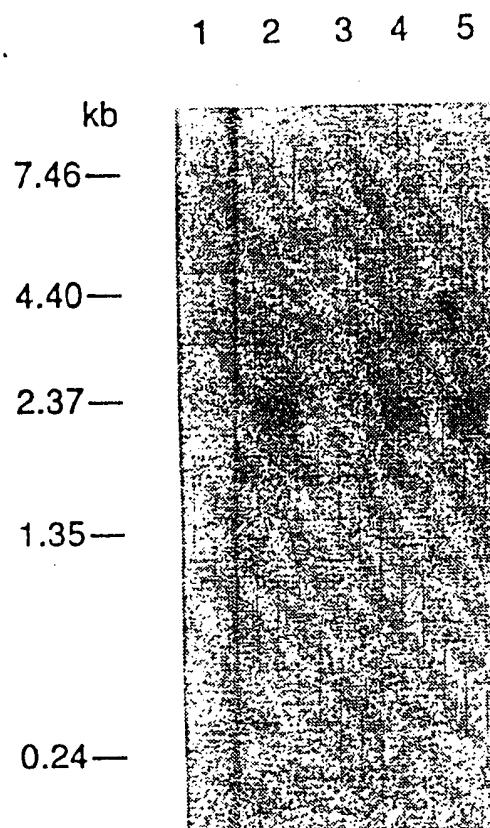


FIG. 6

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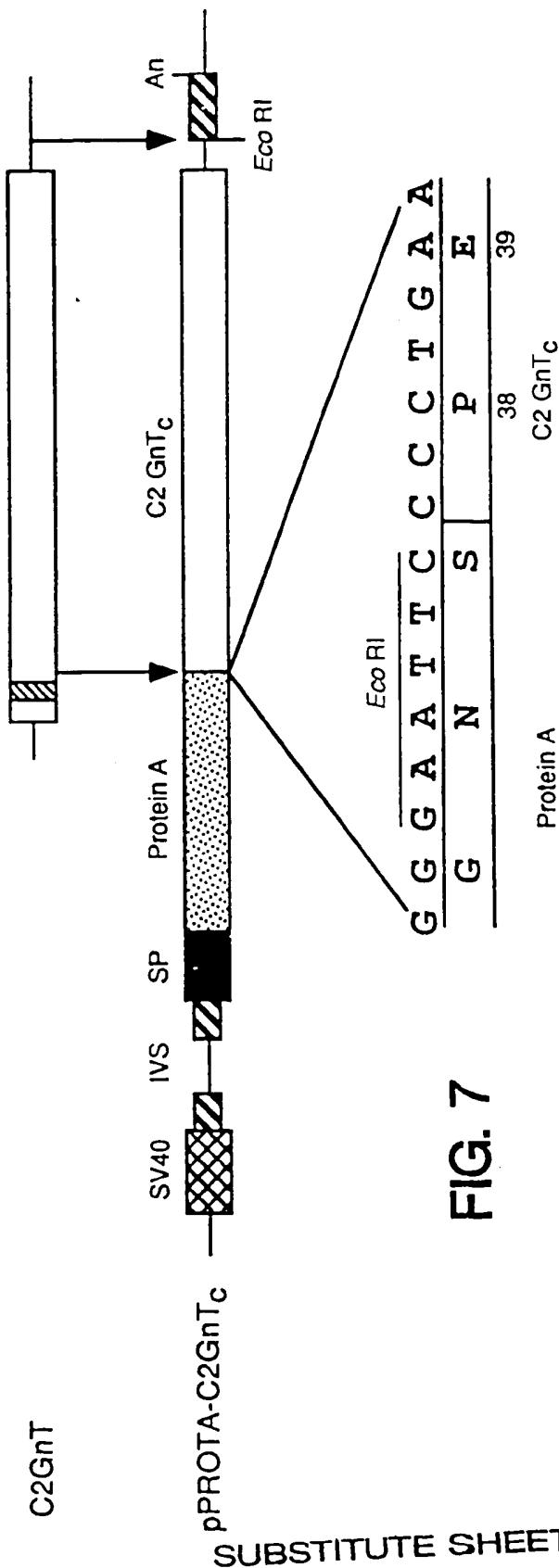


FIG. 7

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09303

A. CLASSIFICATION OF SUBJECT MATTER

IPC(S) :Please See Extra Sheet.

US CL :435/6, 69.1, 193, 240.1, 252.3, 320.1; 530/395, 536/23.2, 23.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 193, 240.1, 252.3, 320.1; 530/395, 536/23.2, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Embase, Pascal, Derwent World Patent Index

search terms: c2gnt, acetylglucosaminyltransferase, core 2, glucosaminyltransferase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings of the National Academy of Sciences USA, Vol. 86, issued February 1989, A. Pallant et al., "Characterization of cDNAs encoding human leukosialin and localization of the leukosialin gene to chromosome 16", pages 1328-1332, see the entire document.	9-15
Y	Journal of Biological Chemistry, Vol. 266, No. 35, issued 15 December 1991, P. A. Ropp et al., "Mucin biosynthesis: Purification and characterization of a mucin beta-6N-acetylglucosaminyltransferase", pages 23863-23871, see the entire document.	1-8, 16-25

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)		
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed	"g"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 December 1993	29 DEC 1993

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer ERIC GRIMES Telephone No. (703) 308-0196
Facsimile No. NOT APPLICABLE	<i>R. Keyya Jr</i>

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/09303

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Blood, Vol. 77, No. 7, issued 01 April 1991, O. Saitoh et al., "T-lymphocytic leukemia expresses complex, branched O-linked oligosaccharides on a major sialoglycoprotein, leukosialin", pages 1491-1499, see the entire document.	1-8, 16-25
Y	Tetrahedron, Vol. 45, No. 17, issued 1989, E. J. Toone et al., "Enzyme-catalyzed synthesis of carbohydrates", pages 5365-5422; see pages 5409-5415.	1-8, 16-25
Y	Molecular and Cellular Biology, Vol. 11, No. 2, issued February 1991, D. F. Stern et al., "Spk1, a new kinase from <i>Saccharomyces cerevisiae</i> , phosphorylates proteins on serine, threonine, and tyrosine", pages 987-1001, see the entire document.	16-24
Y	Molecular and Cellular Biology, Vol. 8, No. 12, issued December 1988, S. Kornbluth et al., "Novel tyrosine kinase identified by phosphotyrosine antibody screening of cDNA libraries", pages 5541-5544, see the entire document.	16-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09303

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

C07K 13/00; C12N 1/21, 5/16, 9/10, 15/12, 15/54, 15/63, 15/79; C12P 21/00; C12Q 1/68

